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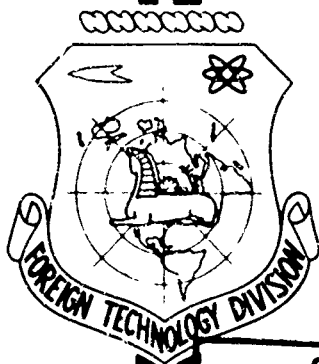
# TRANSLATION

BIOLOGICAL PROPERTIES OF THE NIIEG TULAREMIA  
VACCINAL STRAINS

By

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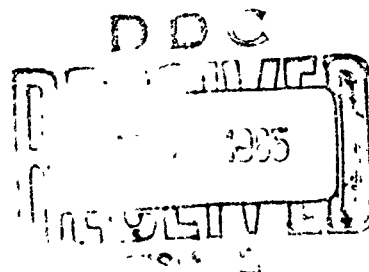
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## UNEDITED ROUGH DRAFT TRANSLATION

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## BIOLOGICAL PROPERTIES OF THE NIEG TULAREMIA VACCINAL STRAINS

L. V. Sirotyn

### Summary

A study was made of the biological properties of the NIEG tularemia vaccinal strains Nos. 10, 33, and 53 as compared to the standard Gaisky's No. 15 vaccinal strain. The properties of the NIEG strain showed no change after a 10-year storage in dried condition without any transfers. Strains Nos. 10 and 53 are harmless for guinea pigs and are highly immunogenic. Strain No. 53 possesses a reduced immunogenicity. The author recommends strain Nos. 10 and 53 as a reserve for the production of dry live tularemia vaccine.

For the purpose of a specific prophylactic for tularemia in the USSR for about twenty years one has successfully used the live tularemia vaccine of Gayskiy and El'bert. Originally for the production of this vaccine one used Gayskiy's vaccine strains No. 15 and muskrat-4. However, in the case of the second one of these soon the immunogenicity went down to such an extent that one came to drop it from production. In the course of some years there were also revealed some changes in the properties of the strain No. 15.

The experimental investigations of Motornaya (1953) and Yemel'yanova (1957) established the possibility of restoring the lowered immunogenicity of vaccine strains by passing through another host animal susceptible to tularemia - white mice or guinea pig. Along with this in the Institute of Epidemiology and

Hygiene (NIEG) there were extracted, studied, and introduced into practice the new tularemia strains Nos. 10, 33, and 53, which in the course of a number of years have been used in the production of live dry tularomia vaccine NIEG along with Gayskiy's strain No. 15 (Faybich and Tamarine, 1946).

In 1950 the vaccine strains NIEG, for the purpose of improving and stabilizing their immunogenicity were passed ten times through the testicles of guinea pigs (Motornaya, 1953). Standard specimens of these strains, prepared in 1952 in the form of dry lyophilic cultures in a saccharogelatin medium were preserved without repassing for ten years at 2 - 10°. The aim of our work was to study the biological properties of the indicated standard cultures of vaccine tularemia strains NIEG for the purpose of learning about the possibility of using them in the future for the production of live vaccine.

For the study there were taken four strains: strain NIEG NO. 10, obtained in 1944 by selecting nonvirulent preserved cultures (Faybich and Tamarina, 1947), strain NIEG No. 33, obtained by Faybich, Saltykov, and Tamarina in 1945 also by selecting preserved strains with lowered virulence, strain NIEG No. 53, extracted by Saltykov in 1947 by selecting colonies from cultures of the virulent strain No. 3, and the strain No. 15 of Gayskiy restored - standard culture 1962 checked by the commission in 1962 and used at the present time in the production of live tularemia vaccine. As tests for the comparison of the biological properties of the vaccine strains there were studied: the cultural-morphological properties, agglutinability, residual virulence for white mice, inoculation capacity in guinea pigs, immunogenicity in experiments on mice and guinea pigs, and harmlessness for guinea pigs. Dry standard cultures were seeded in a yolk medium. In the experiment there were taken 48-hour cultures of the second generation on this medium.

All the strains were typical as to the morphology of the cells, tincture

properties, and character of growth on the feeding media (typical growth on the yolk medium and fish-yeast-blood agar with cystine and glucose and absence of growth on simple media). On the fish-yeast-blood agar with cystine and glucose the colonies of "immunogenic" microbes in the case of the strain No. 10 amounted to 98.5%, for No. 53 - 99.9%, for No. 33 - 99%, and in the case of the standard strain No. 15 of Gayskiy - 99.9%. All the strains were agglutinated to the titration standard of tularemia diagnostic serum of the Odessa Institute of Epidemiology and Microbiology (titer 1:2000) and gave a coarsely cottonlike agglutinate, excepting the strain No. 33, the agglutinate of which was finely cottonlike.

With the inoculation of white mice (of the weight 18 - 20 g) with a 100, 1,000, 10,000, 100,000, and 1,000,000 microbe cells the "residual" virulence, i.e., fatality for the mice in the vaccination period similarly for all the doses amounted to : in the case of strain No. 10 - 91%, for No. 33 - 11%, for No. 53 - 59%, and for No. 15 - 30%. With subsequent infection of the survivors in the process of immunization of the mice with 1,000 Dcl of the virulent strain No. 503 (Dcl = 1 microbe cell in accordance with the standard of the State Control Institute) all the mice remained alive during 15 days of observation (Table 1).

The immunogenicity of the strains was checked also on guinea pigs of the weight of 380 - 400 g. With a culture of each strain in doses of 1,000, 10,000, and 100,000 microbes there were vaccinated 5 each of the guinea pigs. Twenty-seven days later the animals were infected with 1,000 Dcl of the virulent strain No. 503. With the vaccination with a culture of the strain No. 33 there died two guinea pigs out of five, having received 10,000 microbe cells, and four out of five of those inoculated with 1,000 microbe cells. All the guinea pigs vaccinated with cultures of the strains Nos. 10, 53, and 15

Table 1

Residual virulence and immunogenicity for white mice of the vaccine strains  
NIIEG

No. of strains	Dose of vaccination (number of microbe cells)	residual virulence		immunogenicity against 1,000 Dcl of virulent strain	
		absolute number of fatalities (numerator) of total no. (denominator)	% of total for all doses	absolute no. of survivors (numerator) of total no. (denominator)	%
10	100	15/20	91	9/9	100
	1 000	18/20			
	10 000	19/20			
	100 000	19/20			
	1 000 000	20/20			
33	100	2/20	11	89/89	110
	1 000	2/20			
	10 000	2/20			
	100 000	0/20			
	1 000 000	5/20			
50	100	5/20	59	41/41	100
	1 000	12/20			
	10 000	9/20			
	100 000	13/20			
	1 000 000	20/20			
15 of Gay-skiy	100	2/20	30	80/80	100
	1 000	7/20			
	10 000	4/20			
	100 000	5/20			
	1 000 000	12/20			

remained alive during the course of 30 days after the infection (Table 2).

For determining the innoculability of the strains there was obtained a suspension of microbes in a physiological solution with optical density corresponding to 1 billion cells and afterwards there was prepared from it a ten-fold dilution. Guinea pigs of the weight of 400 - 450 g were inoculated cutaneously (scarification method) from the dilutions of the suspension of  $10^{-1}$ ,  $10^{-2}$ , and  $10^{-3}$  (2 guinea pigs for each dilution). The inoculation reaction

Table 2

## Immunogenicity of vaccine strains for guinea pigs

Number of strains	Dose of vaccination (number of microbe cells)	Number of survivors (numerator) of total number of infected (denominator) 1 000 Dcl	
		1952 (Motornaya's data)	after 10 years of storage
10	1 000	5/5	5/5
	10 000	10/10	5/5
	1 000 000	5/5	5/5
33	1 000	1/5	1/5
	10 000	10/10	3/5
	1 000 000	5/5	5/5
53	1 000	5/5	5/5
	10 000	10/10	5/5
	1 000 000	5/5	5/5
15 Gayskiy's (Standard 1962)	1 000		5/5
	10 000		5/5
	1 000 000		5/5

in the case of all animals was quite pronounced (infiltrate and hyperemia around the incision of the dimension of 0.5 - 1.2 cm).

The harmlessness of the strains was studied on guinea pigs of the weight of 350 - 400 g by the method of subcutaneous injection of a suspension of microbes in a physiological solution with an optical density corresponding to 1 and 3 billions of microbe cells ( in accordance with the standard of the State Control Institute). For each dose 3 guinea pigs were taken. All the tested strains proved to be harmless.

If one compares our data from the study of vaccine strains NIEG with Motornaya's data (1953), then it becomes clear that in the process of a 10-year preservation in the dry state these strains hardly changed at all; the



degree of residual virulence for white mice and guinea pigs did not increase, and the cultural-morphological properties and the agglutinability were preserved completely.

In summing up on the study of the NIEG vaccine tularemia strains No. 10 No. 33, and No. 53 in comparison with the standard strain No. 15 of Gayskiy it has been established that the strains Nos. 10 and 53 with heightened residual virulence for white mice proved to be harmless to guinea pigs of the weight of 350 - 400 g in the massive dose of 3 billion microbe cells. Besides this, the vaccine prepared at the same time from these strains with abundant application to persons corresponded with the requirements as to the reaction (Zlatovskiy with co-authors, 1947; Olsuf'yev, 1953). By all the rest of the indices these strains also fully corresponded to the requirements prescribed for vaccine strains. Consequently one can recommend them in the role of preserves for the production of line tularemia vaccines.

The strain No. 33, according to Motornaya's data had a lower immunogenicity. The rest of the vaccine strains and formed in the agglutination reactions a fine cottonlike agglutinate. According to our data it also, as to immunogenicity and agglutinability, surpassed the strains Nos. 10, 53, and 15. In this connection we consider the strain No. 33 to be one that is feasible to use in production.

The strains Nos. 10, 33, and 53 preserved all their qualities after 10 years of storage in the dry state. This can be explained by the high quality of the drying process, as well as by the stability of the strains themselves, which they acquired, apparently, in the process of intratesticular passage through the organisms of guinea pigs. Such a stability merits attention and requires further study in the process of preserving these strains on nourishing media and in passing through the organisms of animals.

### Conclusions

1. The NIIEG vaccine tularamia strains Nos. 10, 33, and 53 practically preserved their biological characteristics in the process of a 10-year storage in the dry state ( in a sucrose-yolk medium).

2. The NIIEG vaccine strains Nos. 10 and 53 are harmless for guinea pigs and are highly immunogenic in experiments on whitemice and guinea pigs, but possess heightened residual virulence for white mice as compared with the standard strain No. 15 of Gayskiy.

3. The NIIEG vaccine strains Nos. 10 and 53 both as to their biological properties revealed at the present time and by experience with their wide practical application in the past can be recommended for the production of live vaccines.

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